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Parathyroid-independent change in renal handling of phosphate in hyperthyroid rats

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Parathyroid-independent change in renal handling of phosphate in hyperthyroid rats. Patients with hyperthyroidism have a plasma concentration and a tubular reabsorption of inorganic phosphate that is higher than that found in normal subjects. These alterations found in hyperthyroidism have been attributed to reduced parathyroid activity, which may result from the moderate rise in plasma calcium. In the first study, we observed that hyperthyroidism (HT) induced by injecting levothyroxine (T_4) (50 μg/100 g of body wt, i.p., daily) to parathyroidectomized (PTX) rats led to an increased concentration of plasma phosphate, whereas hypothyroidism achieved by removing the thyroid gland led to a decreased concentration of plasma phosphate. HT also increased plasma phosphate in PTX and hypophysectomized rats. In the second study, we investigated whether the T_4-induced rise in plasma phosphate was associated with a change in the renal handling of phosphate. Eight days after performing PTX, we induced HT by injecting T_4 at the above-mentioned dose for 10 days. During this period, HT-PTX rats either were pair-fed with euthyroid-PTX counterparts or were fed ad lib but with the same phosphate intake as that given to the other animals. Then, the tubular capacity to reabsorb phosphate was assessed by clearance study. The results showed that in PTX rats HT led to an increase in the capacity to reabsorb phosphate without changing the urinary excretion of cAMP per milliliter of glomerular filtrate. The effect of HT on the tubular phosphate transport was related neither to the caloric intake of the animals nor to the variations in net intestinal phosphorus absorption. It is concluded that thyroxine can influence the renal handling of phosphate and, thus, the level of plasma phosphate independent of parathyroid hormone.

Modification indépendante de l'hormone parathyroïdienne du comportement rénal du phosphate chez les rats hyperthyroïdiens. Les malades atteints d'hyperthyroïdie ont une concentration plasmatique et une réabsorption tubulaire de phosphate inorganique supérieures à celles des sujets normaux. Ces modifications ont été attribuées à une diminution de l'activité parathyroïdienne qui pourrait être la conséquence de l'augmentation modérée du calcium plasmatique qui est observée au cours de l'hyperthyroïdie. Au cours d'une première étude, nous avons observé que chez des rats parathyroïdectomisés (PTX) l'hyperthyroïdisme (HT) obtenu par l'injection quotidienne de levothyroxine (T_4) (50 μg/100 g de poids corporel) était accompagné d'une augmentation du phosphate plasmatique alors que l'hypothyroïdisme obtenu par l'ablation de la glande thyroïde aboutissait à une diminution du phosphate plasmatique. L'hyperthyroïdisme augmente aussi le phosphate plasmatique chez des rats à la fois PTX et hypophysectomisés. Au cours d'une deuxième étude nous avons recherché une modification du comportement rénal du phosphate associée à l'augmentation du phosphore plasmatique induite par la T_4. A partir du huitième jour de la parathyroïdectomie, l'hyperthyroïdie a été déterminée par l'injection de T_4, aux mêmes doses que ci-dessus, pendant 10 jours. Au cours de cette période les rats HT-PTX ont été nourris soit de façon appariée avec leurs homologues PTX euthyroïdiens, soit ad libitum mais avec le même apport de phosphore que les autres animaux. La capacité tubulaire de réabsorption de phosphate a été alors évaluée par une étude de clearance. Les résultats montrent que chez les rats PTX l'hyperthyroïdie détermine une augmentation de la capacité de réabsorption de phosphate sans qu'il y ait de modification de l'excrétion urinaire de cAMP rapportée au ml de filtrat glomérulaire. L'effet de HT sur le transport tubulaire de phosphate ne peut être mis sur le compte ni de l'apport calorique, ni des variations de l'absorption intestinale de phosphate. La conclusion est que la thyroxine peut influencer le comportement rénal du phosphate et de ce fait la concentration plasmatique de phosphate, indépendamment de l'hormone parathyroïdienne.

Patients with hyperthyroidism usually display an increase in concentration of plasma inorganic phosphate [1–6]. This rise in concentration of plasma phosphate could be explained, at least in part, by an increase in tubular reabsorption of phosphate. Indeed, an augmentation in the maximal tubular capacity of phosphate reabsorption has been observed in patients who are thyrotoxic [6, 7]. This renal effect has been attributed [6, 8–11] to a decreased secretion of parathyroid hormone (PTH), which could be explained by an increase in serum calcium secondary to the thyroxine-induced stimulation of bone resorption [12, 13]. In hyperthyroidism, however, a decrease in PTH secretion has not been documented unequivocally [14, 15]. Our initial purpose,

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therefore, was to investigate whether a change in thyroidal status would alter plasma inorganic phosphate in parathyroidectomized (PTX) rats. After ascertaining this, we then studied the renal handling of inorganic phosphate in experimental hyperthyroidism induced by administering levothyroxine to parathyroidectomized rats.

Methods

Protocol 1

Influence of thyroxine on plasma phosphate, calcium, and magnesium in rats with and without parathyroid glands. The concentrations of plasma phosphate, calcium, and magnesium were determined in hypothyroid, euthyroid, and hyperthyroid animals, both in the presence (autotransplantation of parathyroid glands) and in the absence (surgical parathyroidectomy) of endogenous PTH secretion. Male Wistar rats, each weighing about 50 g, were used. Thyroidectomy was performed, with care that the recurrent laryngeal nerve was not damaged. Any possible thyroid remnants in thyroidectomized animals were destroyed by injecting iodine 131 (50 to 100 μCi/100 g of body wt) 2 days after the operation. Control (euthyroid) animals were sham-operated. Three weeks after the operation, the serum concentration of thyroxine (T_4) was less than 2.0 ng/100 ml in all the thyroidectomized animals, compared to 7.5 ± (SEM) 0.4 in control rats. The protein-bound iodine (PBI) was found to be 2.9 ± 0.2 μg/100 ml in thyroidectomized rats, compared to 6.5 ± 0.3 μg/100 ml in control animals.

At the time of thyroidectomy (or sham-operation), the parathyroid glands either were removed surgically (PTX animals) or were autotransplanted into the lateral muscles of the neck. Parathyroidectomy was considered to be adequate when plasma calcium concentration fell by more than 0.65 mM after a 12-hour fasting period. Two weeks after surgery, T_4 (50 μg/day i.p.) or its solvent vehicle was given for 14 days. During this period, the food intake of the animals was controlled strictly. The diet (Altromin C-1000) contained, per 100 g of diet: 1.34 g of calcium, 0.93 g of phosphorus, 100 IU of vitamin D_3, and 320 kcal of energy. Two groups of euthyroid animals were pair-fed with either hypothyroid (TX) or hyperthyroid animals. Seven days after starting the daily administration of T_4, the PBI concentration had risen above a value of 25 μg/100 of plasma in all animals. At the end of the 14-day treatment period, and after the animals were fasted overnight, we obtained blood samples, by puncturing the orbital plexus, between 8 A.M. and 9 A.M., for the determination of plasma concentrations of inorganic phosphate, calcium, and magnesium. Since no significant difference in the plasma levels of these electrolytes was found between the two euthyroid groups, these values are pooled ("Results," Table 1).

Protocol 2

(A) Influence of thyroxine on the renal handling of phosphate in parathyroidectomized animals. In this series of experiments, the tubular capacity to transport inorganic phosphate was assessed in euthyroid and hyperthyroid PTX rats. Male Wistar rats, each weighing 170 to 180 g, underwent parathyroidectomies as described. Calcium concentrations were determined in plasma sampled 3 days after surgery. Only animals displaying a plasma calcium concentration lower than 2.0 mM were kept in the study. Eight days after undergoing PTX, the animals were allotted randomly to three groups, and they underwent one of the following treatments for 10 days. *Group 1:* Euthyroid (EU) -PTX rats received daily i.p. injections of the solvent vehicle of T_4. *Group 2:* Hyperthyroid (HT)-PTX rats received daily i.p. injections of T_4 (50 μg/100 g body wt) and were pair-fed with the animals of group 1. *Group 3:* HT-PTX rats received daily i.p. injections of T_4 (50 μg/100 g body wt), were fed ad lib, but were given

Table 1. Influence of thyroid status on plasma concentration of inorganic phosphate, calcium, and magnesium in rats with (+) and without (−) parathyroid glands[a]

	Inorganic phosphate mM		Calcium mM		Magnesium mM	
	+	−	+	−	+	−
Hypothyroid	2.68 ± 0.03[c]	3.84 ± 0.11[b]	2.45 ± 0.03	1.64 ± 0.05	0.91 ± 0.03	0.81 ± 0.02
Euthyroid	2.95 ± 0.05	4.41 ± 0.12	2.42 ± 0.02	1.61 ± 0.04	0.94 ± 0.03	0.89 ± 0.04
Hyperthyroid	3.04 ± 0.05	4.97 ± 0.09[b]	2.39 ± 0.03	1.48 ± 0.05	1.02 ± 0.04	0.95 ± 0.04

[a] All values are means ± SEM obtained in 12 and 9 animals in the groups with (+) and without (−) parathyroid glands, respectively.
[b] $P < 0.01$, compared to the corresponding euthyroid group.
[c] $P < 0.001$, compared to the corresponding euthyroid group.

the same amount of phosphate as that given to group 1 (see below).

All animals had free access to distilled water. Groups 1 and 2 received a diet containing, per 100 g of diet, 1.1 g of calcium, and 1.2 g of phosphate. This diet was prepared by the addition of sodium phosphate and calcium gluconate to a basic diet (Altromin C-1730) containing, per 100 g of diet, 0.2 g of phosphate and 0.1 g of calcium. The food intake was controlled within triplets consisting of one animal of each group. Within each triplet, the HT-PTX animals (group 2) received as much food as the EU-PTX controls (group 1) had consumed the night before. The HT-PTX animals of group 3 could eat food ad lib; the phosphate content of the diet of group 3, however, was adjusted so that their phosphate intake was similar to that recorded the day before in group 1. This was achieved by providing diets containing 0.96, 0.84, or 0.72 g of phosphate per 100 g of diet. These diets were prepared as mentioned above by adding various amounts of sodium phosphate to the same basic diet (Altromin C-1730). The calcium content of these three diets was also fixed at 1.1 g/100 g of diet.

After 10 days of pair-feeding and T_4 treatment (that is, 24 hours before the clearance experiments), we performed subtotal cystectomies on the animals, while they were under light ether anesthesia, to reduce the dead space of the urinary tract. To obviate any influence of circadian rhythm on phosphate excretion, we started all experiments at about 8:30 A.M. The clearance measurements in conscious rats were performed as previously described [16, 17]. For the present study, a first dose (priming) of inulin (0.4 μCi of (methoxy-^{3}H)-inulin, New England Nuclear, with 12.8 mg of unlabeled inulin, Fluka, dissolved in 0.15 M sodium chloride) was injected i.v. in a volume of 0.4 ml. Isotonic solutions containing 5 μCi of (methoxy-^{3}H)-inulin per 100 ml of solution and 1 g of unlabeled inulin per 100 ml of solution and increasing amount of phosphate were infused then at 4 ml/hr. The animals were infused first with 0.15 M sodium chloride for a 90-min equilibration period. Then, a first urine-collection period of 30 min's duration was made, at the end of which a blood sample was taken from a dorsal hind limb vein. The rats were then infused with phosphate at stepwise increasing doses (45-min equilibrations followed by 30-min urine-collection periods): 60 μmoles, 120 μmoles, and 180 μmoles of phosphate per hour. Blood samples were taken again immediately at the end of each urine collection period.

In a second series of experiments, the influence of T_4 on the renal handling of phosphate was studied under marked extracellular volume expansion. This study was made in EU- and HT-PTX rats. The animals were treated and pair-fed like those presented above in groups 1 and 2. The clearance experiments started, as described above, by infusing i.v. a 0.15 M sodium chloride and inulin solution at 4 ml/hr. After a 90-min equilibration period, urine was collected for 30 min. The 0.15 M sodium chloride solution was then infused at 20 ml/hr until the end of the experiment. After a second equilibration period of 60 min's duration, a 15-min urine collection was made. Then phosphate was infused at 60, 120, and 180 μmoles/hr. For each i.v. phosphate load, urine was collected for 15 min after a 45-min equilibration period. At the end of each urine collection, a blood sample was taken as described above. The degree of extracellular fluid volume expansion achieved in this experiment was estimated by weighing the animals at the beginning and end of the isotonic saline infusion. The mean ($\pm$ SEM) increase in body weight was 21.3 $\pm$ 3.8% ($N = 6$) and 19.5 $\pm$ 3.4% ($N = 6$) in the EU and HT rats, respectively. In addition in this series of experiments, the urine samples were collected in chilled test tubes, and aliquots were immediately frozen for later determination of cAMP.

(B) Influence of thyroxine on intestinal phosphorus absorption. Since change in the supply and thus in the intestinal absorption of phosphorus has been shown to markedly alter the renal handling of phosphate [18, 19], the influence of T_4 on this variable was assessed. EU- and HT-PTX rats belonging to groups 1 and 2 were pair-fed and treated as described above for 11 days. Then, the animals were put in individual metabolic cages. T_4 treatment and pair-feeding were continued for another 6-day period. During the last 72 hours, the food intake was monitored, and feces and urine samples were collected for phosphorus determination. From these three measurements, the net intestinal absorption and the body retention of phosphorus were calculated.

Analytical methods: Protocol 1. Inorganic phosphate concentration was determined in plasma by the method described by Henry [20], and calcium and magnesium concentrations were by atomic absorption spectrophotometry (Perkin Elmer, Type 290 B). *Protocol 2.* Urinary volume (V) was determined by weighing. The activity of ^{3}H-inulin in plasma and urine was measured in a scintillation spectrometer. Phosphate was determined in diets, plasma, urine, and feces as phosphomolybdate after reduction with 10% ascorbic solution [21]. Sodium concentra-

tion in plasma and urine was determined by flame photometry (EEL, flame photometer, Evans Electroselenica Ltd., Halstead, Essex, England). The diets and feces were analyzed after incineration of the samples and dissolution of the ash in 0.1 M hydrochloric acid. The osmolality of solutions for infusion was measured with an osmometer (Model 3W, Advanced Instruments Inc., Needham Heights, Massachusetts, USA). Urinary cAMP was determined by competitive binding assay with a commercial kit (cAMP assay kit, The Radiochemical Centre, Amersham, England).

Statistical analysis. Significance of difference between groups was assessed by the two-sided Student's *t* test.

Results

Influence of thyroxine on plasma phosphate. In the presence of parathyroid glands, plasma phosphate (Table 1) was significantly lower in hypothyroid (HT) than it was in euthyroid (EU) animals. No significant difference, however, was found between HT and EU rats. In the absence of parathyroid glands, the plasma phosphate level was also significantly lower in hypothyroid than it was in EU rats. Furthermore, in PTX animals, a difference in plasma phosphate between HT and EU rats could be observed. As expected, the phosphate concentration was significantly ($P < 0.01$) higher in PTX animals than it was in the corresponding groups with parathyroid glands. The change in phosphate concentration observed in hypothyroid or HT rats was not associated with any significant alteration in plasma calcium or magnesium concentrations (Table 1).

Influence of thyroxine on the renal handling of phosphate in parathyroidectomized rats. In this series of experiments (Table 2), a trend for an increase in plasma phosphate concentration was noted also in HT animals. Such an elevation could still be observed 2 hours after the outset of the saline infusion (period 1), and it persisted when phosphate was delivered at 60 and 120 μmoles/hr. Nevertheless, the difference achieved statistical significance only when the HT group pair-fed with respect to food (HT-pfF) was compared to the EU animals (Table 2). When the largest phosphate load (180 μmoles/hr) was administered, the difference in plasma phosphate between EU and HT rats was cancelled. During the first three clearance periods, the increased plasma phosphate in HT rats was not associated with a commensurate elevation in urinary excretion of phosphate ($U_{Pi}V$) per milliliter of glomerular filtrate (GF). Hence, tubular reabsorption of phosphate (TRP_i) was higher in HT rats even when factored per milliliter of glomerular filtrate (GF). It is well established that in intact [22] or PTX [18, 23, 24] rats a *stable* maximal phosphate transport capacity (Tm) cannot be observed, since TRP_i per milliliter of GF decreases during the acute infusion of phosphate. The same phenomenon is observed also in this study, not allowing the use of Tm/GF for expressing the phosphate transport capacity. That T_4 actually induced an increase in the tubular capacity

Table 2. Renal handling of inorganic phosphate (P_i) in euthyroid- (EU) and hyperthyroid- (HT) parathyroidectomized (PTX) rats[a]

Period	Infused P_i rate μmole/hr	Group[b]	C_{In} ml/min	FE_{Na} %	$[P_i]$ mM	$U_{Pi}V$ μmoles/ml GF	TRP_i μmoles/min	TRP_i μmoles/ml GF
1	0	EU	1.87 ± 0.12	4.62 ± 0.53	4.22 ± 0.08	0.44 ± 0.08	6.78 ± 0.44	3.78 ± 0.14
		Ht-pfF	2.44 ± 0.20[c]	1.63 ± 0.33[e]	4.99 ± 0.16[d]	0.17 ± 0.07[c]	11.59 ± 0.85[e]	4.81 ± 0.17[e]
		HT-pfP	2.67 ± 0.21[d]	2.70 ± 0.33[c]	4.52 ± 0.29	0.08 ± 0.05[d]	11.85 ± 1.06[e]	4.44 ± 0.27
2	60	EU	1.92 ± 0.15	3.87 ± 0.27	4.66 ± 0.16	0.61 ± 0.06	7.90 ± 0.53	4.06 ± 0.20
		HT-pfF	2.72 ± 0.15[d]	1.87 ± 0.32[c]	5.67 ± 0.12[e]	0.38 ± 0.11	14.45 ± 0.93[e]	5.30 ± 0.17[e]
		HT-pfP	2.52 ± 0.17[c]	1.63 ± 0.23[e]	5.29 ± 0.12[c]	0.18 ± 0.09[d]	12.86 ± 1.06[e]	5.11 ± 0.20[d]
3	120	EU	1.80 ± 0.10	4.21 ± 0.44	5.09 ± 0.17	1.12 ± 0.07	7.09 ± 0.39	3.97 ± 0.21
		HT-pfF	2.49 ± 0.12[c]	2.42 ± 0.28[c]	5.82 ± 0.11[d]	1.03 ± 0.09	11.82 ± 0.54[e]	4.78 ± 0.18[e]
		HT-pfP	2.42 ± 0.15[d]	2.33 ± 0.14[d]	5.65 ± 0.21	0.79 ± 0.09[c]	11.70 ± 0.85[e]	4.86 ± 0.26[e]
4	180	EU	1.93 ± 0.21	4.52 ± 0.47	5.63 ± 0.20	1.75 ± 0.09	7.36 ± 0.76	3.88 ± 0.18
		HT-pfF	2.52 ± 0.14[c]	2.35 ± 0.12[e]	5.92 ± 0.13	1.63 ± 0.05	10.73 ± 0.54[e]	4.29 ± 0.13
		HT-pfP	2.43 ± 0.13	2.54 ± 0.22[d]	5.83 ± 0.18	1.64 ± 0.05	10.19 ± 0.64[c]	4.20 ± 0.21

[a] All values are means ± SEM.

[b] Abbreviations are for tubular reabsorption of phosphate (TRP_i), and pair-fed (pf) with EU-PTX rats for food (F) and phosphorus (P). The daily mean (± SEM) of food intake (g dry weight) measured during the 10 days preceding the clearance study was: in EU, 23.5 ± 0.7; in HT-pfF, 23.4 ± 0.7; in HT-pfP, 29.0 ± 1.6. On the clearance day the body weight (± SEM) was: in EU, 227 ± 4 g; in HT-pfF, 201 ± 4 g; in HT-pfP, 206 ± 6 g. Number of animals: EU, $N = 7$; HT-pfF, $N = 8$; HT-pfP, $N = 6$.

[c] $P < 0.05$, compared to EU group.

[d] $P < 0.01$, compared to EU group.

[e] $P < 0.001$, compared to EU group.

to reabsorb phosphate is illustrated in Fig. 1. Indeed, up to a plasma phosphate concentration of about 5.5 mM, the fractional excretion of phosphate was always lower in HT than it was in EU rats. Note that no apparent difference in the renal handling of phosphate could be detected between the two hyperthyroid groups. This indicates that the T_4-induced change in the tubular capacity to reabsorb phosphate is not related to a difference in the amount of ingested calories. As expected [25, 26], the GFR was markedly increased in both HT groups as compared to the EU animals (Table 2). The fractional excretion of sodium (FE_{Na}) was significantly reduced in hyperthyroid animals (Table 2). These differences in GFR and FE_{Na} were observed throughout the experiment.

Influence of thyroxine on the renal handling of phosphate during extracellular volume expansion. The results of FE_{Na} presented above suggested that the T_4-induced change in the renal handling of phosphate was secondary to alteration in extracellular volume (ECV) and tubular reabsorption of sodium. To investigate whether a marked increase in ECV and FE_{Na} could cancel the effect of T_4 on the renal handling of phosphate, we studied the tubular response to phosphate infusion in rats receiving a solution of isotonic saline at 20 ml instead of 4 ml/hr. In EU as in HT-PTX rats, increasing the rate of saline infusion decreases plasma phosphate without significantly changing FE_{P_i} (Fig. 2). Such an observation is consistent with the large number of observations [22, 27–32] indicating that ECV expansion reduces the tubular capacity to reabsorb phosphate. This effect of ECV expansion is evident also when the values of $U_{P_i}V$/ml GF in either EU or HT-PTX animals are compared (Table 3) at similar plasma phosphate concentrations before (period 1) and during marked ECV expansion (period 3). With respect to the effect of T_4, Table 3 shows that the difference in TRP_i/ml GF is not abolished under marked ECV expansion. Indeed, in these circumstances, FE_{P_i} remains smaller in HT-PTX than it does in EU-PTX animals over a wide range of plasma phosphate (Fig. 2). The difference in FE_{Na} between EU- and HT-PTX rats, however, also was maintained throughout this experiment, with the exception of period 2 (Fig. 3, Table 3). As a consequence of the increased GFR, the diminutions in the FE_{Na} and FE_{H_2O} were not associated with concomitant reductions in their absolute excretion rate in HT animals. This is illustrated in Fig. 3, where the fractional and absolute excretion of sodium or water are plotted against their respective cumulative infused load.

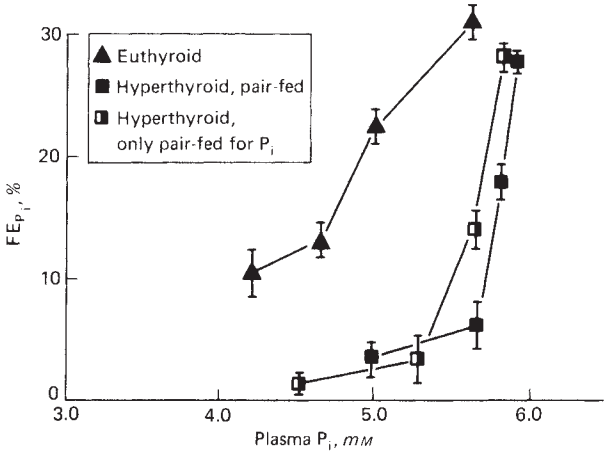

Fig. 1. *Influence of hyperthyroidism on the renal handling of inorganic phosphate (P_i) in parathyroidectomized rats.* Hyperthyroidism was induced by injecting thyroxine (T_4) at the dose of 50 μg/100 g body wt, i.p., for 10 days. Then fractional excretion of phosphate (FEP_i) was measured at endogenous and increasing plasma phosphate concentration altered by infusing phosphate at 60, 120, and 180 μmoles/hr. Values are the means ± SEM. See text and Table 2 for further details.

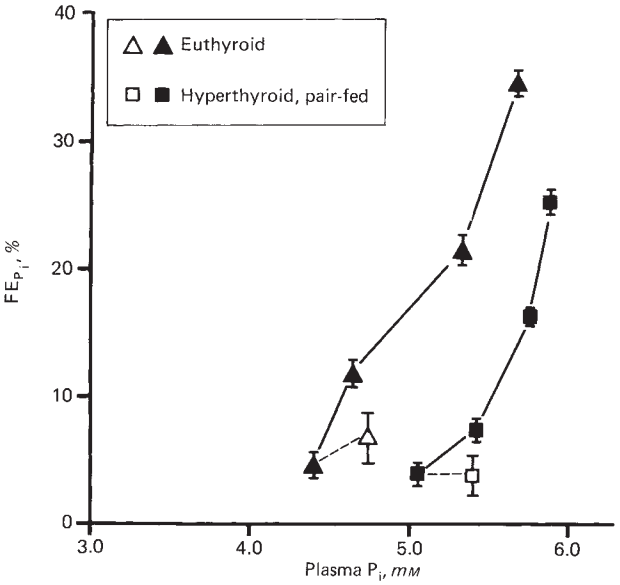

Fig. 2. *Maintenance of the influence of hyperthyroidism on the renal handling of inorganic phosphate (P_i) of parathyroidectomized rats during acute extracellular volume expansion.* Hyperthyroidism was induced by injecting thyroxine (T_4) at the dose of 50 μg/100 g body wt, i.p., for 10 days. Then the fractional excretion of phosphate (FE_{P_i}) was measured first at endogenous plasma phosphate concentration when the animals were infused i.v. with 0.15 M sodium chloride solution delivered at 4 (△□) and 20 (▲■) ml/hr. Under this latter rate of infusion, plasma phosphate concentration was raised by infusing phosphate at 60, 120, and 180 μmoles/hr. Values are means ± SEM. See text and Table 3 for further details.

Table 3. Renal handling of inorganic phosphate and urinary cAMP excretion during extracellular volume expansion in euthyroid- (EU) and hyperthyroid- (HT) parathyroidectomized (PTX) rats[a]

Period	Infusion rate: Fluid ml/hr	Infusion rate: P_i μmole/hr	Group	C_{In} ml/min	FE_{Na} %	$[P_i]$ mM	$U_{P_i}V$ μmole/ml GF	TRP_i μmole/min	TRP_i μmoles/ml GF	$U_{cAMP}V$ pmoles/ml GF
1	4	0	EU	1.81 ± 0.12	3.81 ± 0.41	4.72 ± 0.12	0.316 ± 0.095	7.88 ± 0.23	4.40 ± 0.15	26.2 ± 0.9
			HT-pfF	2.23 ± 0.14[b]	2.14 ± 0.30[c]	5.38 ± 0.16[b]	0.218 ± 0.079	11.48 ± 0.78[c]	5.17 ± 0.17[c]	22.2 ± 4.1
2	20	0	EU	1.70 ± 0.18	7.36 ± 1.05	4.40 ± 0.07	0.211 ± 0.046	7.11 ± 0.79	4.19 ± 0.08	26.5 ± 4.2
			HT-pfF	2.40 ± 0.18[b]	7.33 ± 0.66	5.05 ± 0.10[d]	0.207 ± 0.056	11.59 ± 0.89[c]	4.84 ± 0.10[d]	29.2 ± 1.9
3	20	60	EU	1.58 ± 0.16	16.46 ± 0.50	4.64 ± 0.08	0.559 ± 0.054	6.42 ± 0.63	4.08 ± 0.05	20.2 ± 2.8
			HT-pfF	2.39 ± 0.12[c]	9.92 ± 0.69[d]	5.42 ± 0.12[d]	0.409 ± 0.054	11.95 ± 0.71[d]	5.01 ± 0.16[d]	33.7 ± 4.8[b]
4	20	120	EU	1.71 ± 0.20	16.08 ± 0.88	5.32 ± 0.06	1.153 ± 0.068	7.30 ± 0.75	4.17 ± 0.09	100.8 ± 9.0
			HT-pfF	2.35 ± 0.18[b]	13.38 ± 0.80[b]	5.75 ± 0.10[d]	0.945 ± 0.037[b]	11.33 ± 0.90[c]	4.81 ± 0.09[d]	87.8 ± 8.6
5	20	180	EU	1.47 ± 0.21	19.06 ± 0.82	5.66 ± 0.08	1.975 ± 0.050	5.44 ± 0.81	3.69 ± 0.11	63.0 ± 6.6
			HT-pfF	2.12 ± 0.15[d]	13.14 ± 0.23[d]	5.87 ± 0.15	1.516 ± 0.047[d]	9.25 ± 0.75[c]	4.35 ± 0.14[c]	66.3 ± 7.2

[a] See Table 2 for definition of abbreviations. Number of animals were: EU, $N = 6$; HT-pfF, $N = 6$. On the clearance day the body weight (± SEM) was: in EU, 190 ± 3 g; in HT-pfF, 164 ± 3 g.
[b] $P < 0.05$, compared to the EU group.
[c] $P < 0.01$, compared to the EU group.
[d] $P < 0.001$, compared to the EU group.

Influence of thyroxine on urinary cAMP. Table 3 also indicates that the effect of T_4 on the renal handling of phosphate is not associated with a difference in the excretion rate of cAMP monitored per milliliter of glomerular filtrate (GF). Note that in this experiment $U_{cAMP}V$/ml GF appears to increase significantly in both EU and HT rats in response to phosphate, or isotonic saline infusion, or both.

Influence of thyroxine on intestinal phosphorus absorption. To determine whether a change in the renal handling of phosphate could be the result of a primary alteration in the intestinal absorption, or in the body retention of phosphorus, or in both, we made a balance study in a group of HT-PTX rats pair-fed with EU-PTX counterparts. No significant difference in net phosphorus intestinal absorption (ingested phosphate minus fecal phosphate) could be detected: HT rats, 91 ± (SEM) 2 mg/day, $N = 7$; EU rats, 88 ± 2 mg/day, $N = 7$. Urinary phosphate excretion was not significantly altered by T_4 administration: HT rats, 49 ± 4 mg/day; EU rats, 42 ± 3 mg/day. Thus, under these experimental conditions, T_4 did not change the overall phosphorus retention: HT rats, 42 ± 2 mg/day; EU rats, 46 ± 2 mg/day.

Discussion

Influence of thyroxine on plasma inorganic phosphate. Our study shows that thyroxine (T_4) can increase the concentration of plasma phosphate in the absence of endogenous parathyroid hormone (PTH). In fact, the rise in plasma phosphate in response to T_4 administration is even more pronounced in the absence than it is in the presence of endogenous PTH. In view of these experimental findings, it appears unlikely that the elevated plasma phosphate concentrations observed in hyperthyroid patients [1-3, 5, 6, 33] result merely from a reduction in PTH secretion, which until now usually has been assumed [6, 8, 11]. Our study also shows that removal of the thyroid gland leads to a decrease in plasma phosphate in the parathyroidectomized (PTH) animal. This suggests that thyroid hormones influence plasma phosphate not only in pharmacologic but also in physiologic amount. The hyperphosphatemic effect of T_4 is not associated with any consistent change in the concentration of plasma calcium and magnesium. It does not seem to require the presence of growth hormone or other pituitary factors. Indeed, in hypophysectomized (HPX) and PTX rats, administration of T_4 (50 μg/day, i.p., for 14 days) also induced a rise of the phosphatemia: HPX-PTX + T_4, 3.71 ± (± SEM) 0.16 mM, $N = 9$; HPX-PTX + solvent vehicle 2.67 ± 0.09 mM, $N = 9$; $P < 0.01$. The reported balance study made in pair-fed animals indicates that neither the net intestinal absorption nor the urinary excretion of phosphate appear to be altered under the influence of long-term T_4 administration. Therefore, a change in the extrarenal handling of phosphate does not seem to play any significant role in the elevation of plasma phosphate. It appears more likely that the influence of T_4 on plasma phosphate of PTX animals is directly related to its action on the tubular handling of phosphate.

Influence of thyroxine on the renal handling of

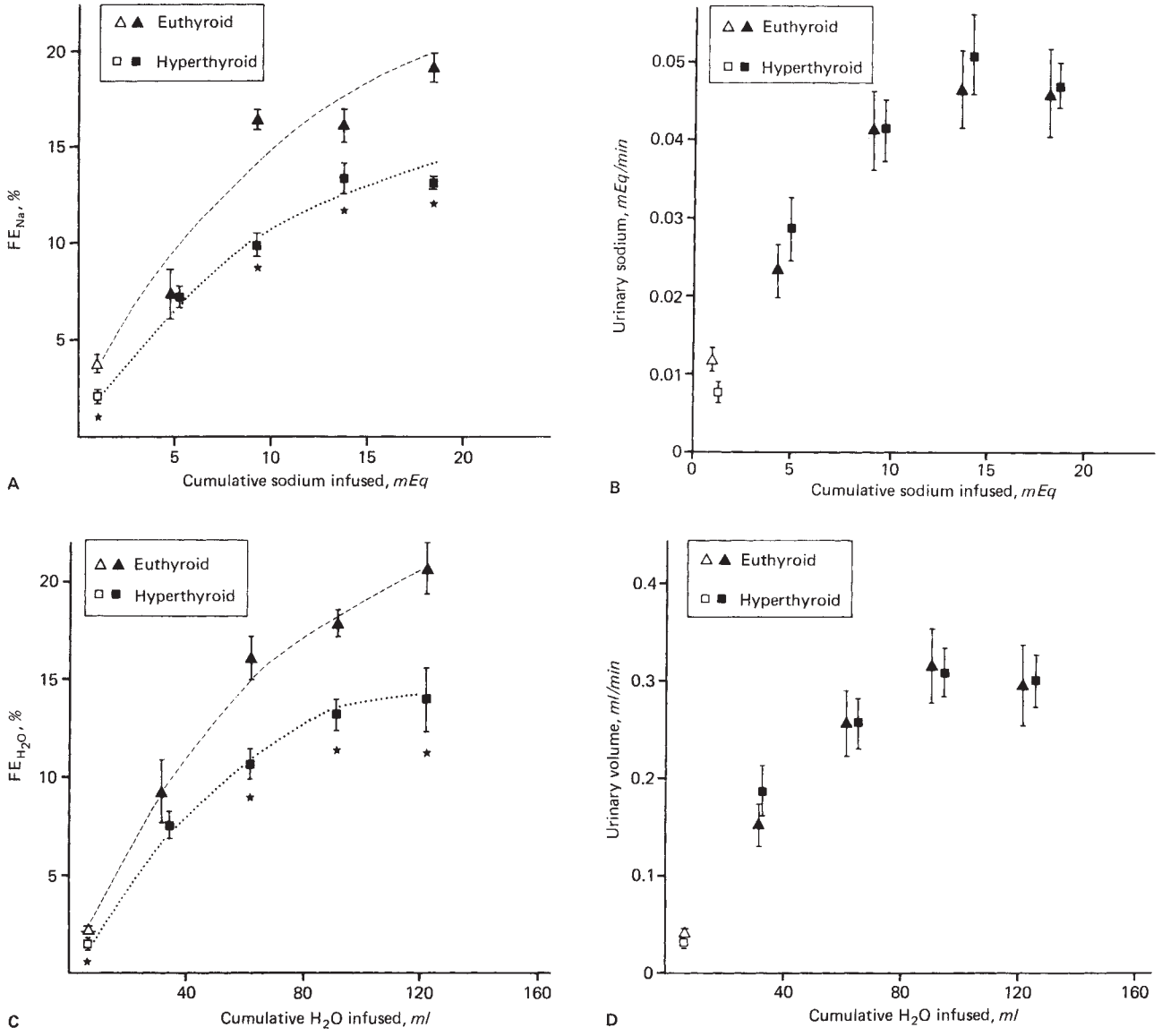

Fig. 3. *Influence of hyperthyroidism on the natriuretic and diuretic response to isotonic saline infusion.* The fractional (panels A and C) and absolute (panels B and D) amount of sodium and water excreted is plotted as a function of the cumulative sodium and water load infused during the acute administration of a 0.15 M sodium chloride solution. Hyperthyroidism appears to alter the fractional but not the absolute amount of sodium and water excreted. Asterisks (*) denote $P < 0.05$. Values are means ± SEM. See text and Table 3 for further details.

inorganic phosphate. Our study demonstrates that the stimulatory effect of T_4 on the tubular capacity to reabsorb phosphate can be elicited in PTX animals. This strongly suggests that the increased Tm_{P_i} (maximal phosphate transport capacity) previously observed in thyrotoxic patients [6, 7] is a PTH-independent process. In fact, our observation (Table 1) indicates that the T_4-induced rise in plasma phosphate is more pronounced in PTX than it is in intact animals, suggesting that the presence of PTH would tend to attenuate the stimulatory action of T_4 on the tubular phosphate reabsorptive capacity. The mechanism whereby T_4 promotes a change in the renal handling of phosphate in PTX rats does not seem to be related to an alteration in the adenyl cyclase system. Indeed, in our experiments, $U_{cAMP}V$/ml GF was not diminished in hyperthyroid-PTX rats. The significant increase in $U_{cAMP}V$/ml GF observed in response to phosphate infusion in both EU and HT-PTX groups is in agreement with a recent finding obtained by Webb et al. [34] in thyroparathyroidectomized rats. From our present ex-

periment, however, it is difficult to conclude that the rise in $U_{cAMP}V$/ml GF results merely from the elevation in plasma phosphate, since the animals were simultaneously submitted to progressive ECV expansion. Another recent study made in thyroparathyroidectomized rats [17] did not reveal any significant increase in cAMP excretion in response to a phosphate infusion similar to that delivered in the present work. In these former circumstances [17], however, the rise in plasma phosphate was not accompanied by a conspicuous increase in expansion and FE_{Na}. Therefore, it would seem appropriate to further assess whether ECV expansion plays any role [35, 36] in the elevation of $U_{cAMP}V$ that can be observed during phosphate infusion in PTX animals.

Among other factors known to influence the renal handling of phosphate, a change in the intake [18, 19] or intestinal absorption of phosphate is probably not implied in view of the results of the balance study. The absolute need for phosphate as determined by growth, anabolism, and bone mineralization may influence the renal handling of phosphate. A previous study [37] showed that HT-PTX animals have an equal rate of longitudinal growth but a diminished rate of weight gain, compared to pair-fed EU-PTX counterparts. No impairment in the mineralization [37] and ash content (Bommer and Ritz, unpublished data) of bone could be detected in the HT-PTX rats. If, in view of the reduced weight gain, however, the need for phosphate would be diminished in hyperthyroidism, one would expect a decrease rather than an increase in the tubular reabsorption of phosphate. Therefore, in this respect only, a relative increase in the phosphate need of the organism could explain the tubular phosphate response to thyroxine. Whether the influence of T_4 on cellular metabolism would increase phosphate requirement and thus lead secondarily to a tubular adaptation remains purely speculative.

A previous study [38] has documented a reduction in the ECV in rats with T_4-induced hyperthyroidism. In the present work the reduced FE_{Na} observed in HT-PTX animals does not seem to be the consequence of a possible reduction in ECV. Indeed the difference in FE_{Na} between EU and HT-PTX rats cannot be cancelled out by infusing large amounts of isotonic saline solution (Fig. 3). Instead of reflecting the response to a reduced ECV, the decrease in FE_{Na} may be interpreted as the consequence of a tubular adjustment to the increased filtered load of sodium [39]. Alternatively, it could be the results of a direct action of T_4 on the tubular reabsorption of sodium. Thyroid hormones have been shown to influence the renal Na-K-ATPase [40], a phenomenon which has been shown to be associated with parallel changes in the tubular reabsorption of sodium [25, 39, 41]. By whichever mechanism FE_{Na} is reduced, the question arises whether influence of T_4 on phosphate reabsorption is secondary to its action on the tubular sodium transport. Under many circumstances, tubular changes of the phosphate transport parallel that of sodium [42, 43]. Recently, studies on brush border vesicles [44] support the concept of a sodium dependence of phosphate transport, at least at the level of the luminal plasma membrane of the proximal tubule. From our experiments, we cannot rule out the possibility that the T_4-induced change in the renal handling of phosphate was secondary to the stimulation of the tubular sodium transport. Indeed, both the sodium and phosphate alterations were maintained during marked ECV expansion (Figs. 2 and 3, Table 2). The change in the renal handling of phosphate, however, was associated with an increase in the phosphatemia. Therefore, the rise in the tubular capacity to reabsorb phosphate cannot be considered merely as an adaptive mechanism tending to compensate for the augmentation in the filtered load of phosphate caused by the increased GFR. We cannot, however, rule out entirely the possibility that the increased GFR in itself may through some unknown mechanism enhance the process of net phosphate reabsorption beyond the rate which is needed to compensate for the increase in filtered load. Presently, no evidence has been reported which would support that GFR itself might have such an effect on the tubular phosphate transport system. Further experiments should be aimed at ascertaining whether the parathyroid-independent action of thyroxine on the renal handling of phosphate can be dissociated from its effect on GFR and the tubular sodium transport. Its site of action along the nephron should be localized and the nature of its effect characterized. This may be accomplished by experiments that could answer the question of whether thyroxine affects the net phosphate reabsorption by altering the transport system bound to the luminal or contraluminal membrane [44] or by modifying the driving forces influencing this system. Yet another possibility would be that thyroxine is changing the paracellular permeability of the tubular epithelium. Finally, the consideration that vitamin D_3 or one of its metabolites may play a mediating role in the action of thyroxine on the renal

transport of phosphate should be included also in future investigations.

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